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# Electron Histochemical Demonstration of Acid Phosphatase Activity in the Motoneurons of the Cervical Spinal Cord of Rat during Axonal Reaction

by

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Acid phosphatase activity in terms of lysosomes in the central nervous system has been investigated at the level of light microscopic histochemistry by several authors (BARRON, '61<sup>1)</sup>; BECKER & BARRON, '61<sup>4)</sup>; BARRON & SKLAR, '61<sup>2)</sup>; BARRON & TUNCBAY, '62<sup>3)</sup>; OGAWA et al., '60<sup>9)</sup>; OGAWA et al., '61<sup>10)</sup>).

NOVIKOFF ('59)<sup>7)</sup> found enlargement of lysosomes in the cells of the proximal tubules in experimental renal hydronephrosis, and for such enlarged lysosomes he designated the term "cytolysome". Barron attempted to study cytochemically response of lysosomes to injury of nervous tissue and reported that enlarged droplets rich in acid phosphatase activity are demonstrated in the necrotizing neurons in the brain injured by diphtheria toxin (BARRON, '61<sup>1)</sup>). The similar findings in the neurons have also been observed during the early phase of both postmortem autolysis and necrobiosis in the anoxic and anoxic-ischemic encephalopathy (BECKER & BARRON, '61<sup>4)</sup>) and also during axonal reaction (BARRON & SKLAR, '61<sup>2)</sup>).

In the present investigation attempts were made to demonstrate electron histochemically response of acid phosphatase activity in the motoneurons of the cervical spinal cord to axonal reaction.

The cervical spinal cords of Wistar rats, weighing 120 to 200g, were used. Section of the brachial nerve was performed through posterior paraspinous approach. Animals were sacrificed seven and fourteen days after operation.

GOMORI's lead acetate method for acid phosphatase (GOMORI, '52<sup>6)</sup>) was essentially used in cold formol-calcium fixed frozen sections. Substrate-free incubating media were taken for controls. After incubation specimens were postfixated in Palade's buffered osmium tetroxide and processed according to the routine procedure for the observation by the electron microscope (OGAWA et al., '62<sup>11)</sup>).

In the present study deposits of electron opaque lead phosphate indicating acid phosphatase activity were positive in dense bodies (lysosomes) in the perikaryon (Fig. 1). In some dense bodies precipitates of lead phosphate were found in their limiting membrane and also scatteredly in the bodies. The smooth membranes, presumably the membrane

components comprising the Golgi apparatus were also positive for enzymatic activity. However, no mitochondria showed enzymatic activity. NOVIKOFF & ESSNER ('62)<sup>8)</sup> described that lysosomes are produced by dilatation and separation of the Golgi cisternae. OSINCHAK (1964)<sup>12)</sup> also reported that acid phosphatase activity is localized to certain Golgi cisternae, surrounded by a single membrane.

Though size of enzymatically active dense bodies in the motoneurons did not show any appreciable change during axonal reaction, number of dense bodies seemed to be increased markedly (Figs. 2 & 3). The electron density of dense bodies itself was also much higher in axonal reaction than in normal controls. This finding is due to abundance of lead phosphate precipitates indicating the increased enzymatic activity within dense bodies. Occasionally vacuolated dense bodies were also observed. Enlarged lysosomes (cytolysome) observed under the light microscope by BARRON & SKLAR ('61)<sup>2)</sup> were not seen in the present investigation carried at the level of electron microscope.

In the present investigation it was observed that dense bodies markedly increased in number in cervical motoneurons during axonal reaction. This finding may indicate the increased acid phosphatase activity, which is in agreement with the result of biochemical study by BODIAN & MELLORS ('45)<sup>5)</sup>. The increased enzymatic activity may be indicative of increased hydrolytic activity as suggested by NOVIKOFF ('59)<sup>7)</sup>, however, direct involvement of the enzyme with cellular metabolic activity in the motoneurons during the process of axonal reaction can not be excluded.

This study was carried out under the helpful suggestion by Dr. KAZUO OGAWA, Professor of Anatomy, Kansai Medical School, Moriguchi, Osaka, Japan.

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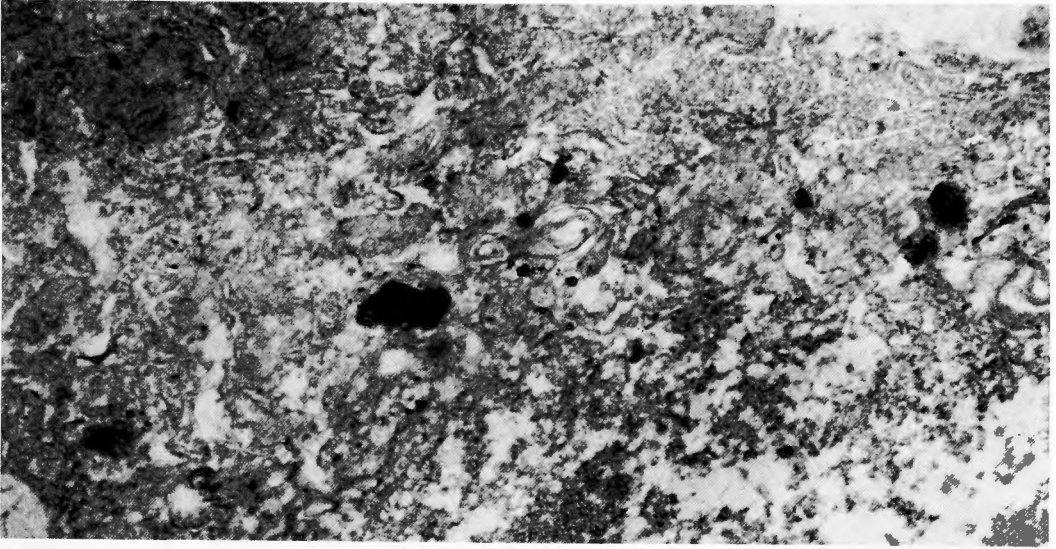


Fig. 1

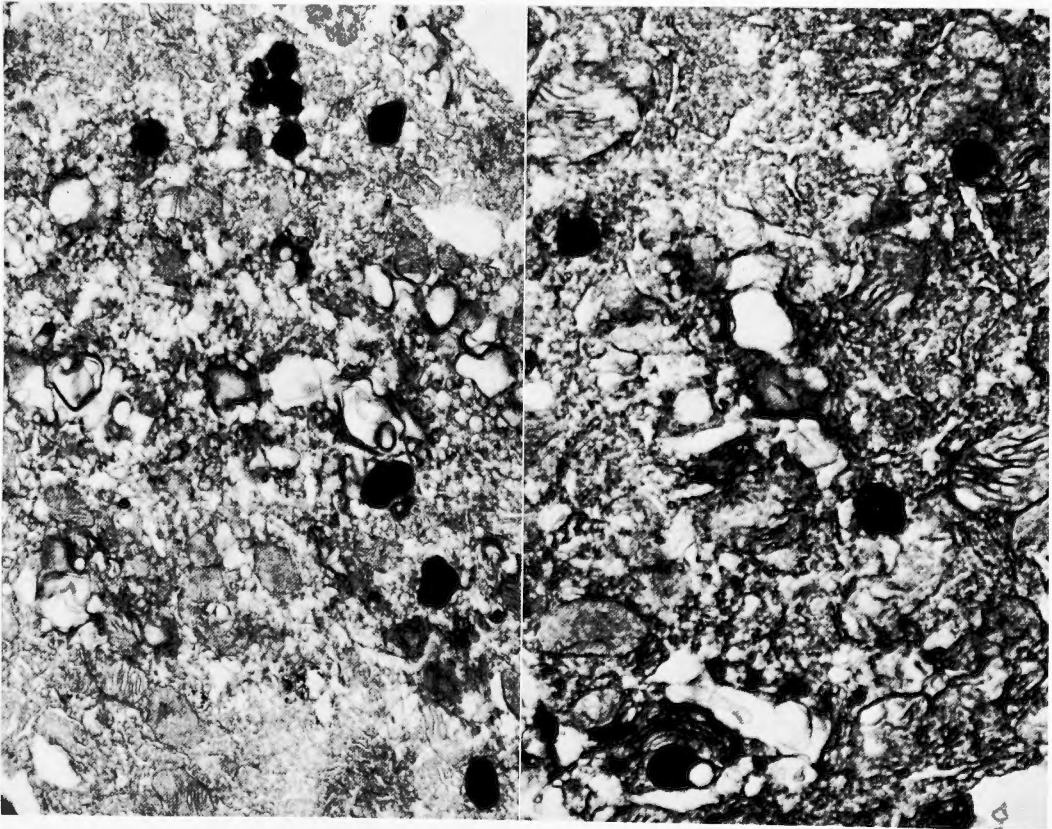


Fig. 2

Fig. 3

**Fig. 1** Deposits of electron opaque lead phosphate showing the acid phosphatase activity are seen in dense bodies in perikaryon of cervical motoneurons in the normal adult rat. 26000×

**Figs. 2 & 3** Cervical motoneurons. Fourteen days after the section of brachial nerve. Note an increase in number of dense bodies. The electron density of bodies is also highly increased.

Fig. 2, 26000× Fig. 3, 29000×

## 和文抄録

# 軸索反応の際に見られるラット脊髄前角細胞内酸性 フォスファターゼ活性の電子顕微鏡学的研究

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田 中 清 介

ラットの上腕神経を切断し、7日及び14日後における頸髄前角細胞を用いた。材料は冷フォルモール・カルシウムで固定し、これを凍結切片とした後、Gomoriの錯酸鉛法で酸性フォスファターゼ活性を検出した。後固定には Palade の緩衝・酸化オスミウム液を使用し、通常の電子顕微鏡標本と同じ過程を経てエボン包埋を行なった。

酸性フォスファターゼは正常脊髄前角細胞内においてもリソゾーム (lysosome) および Gomori 装置を含む滑面小胞体にも認められた。神経切断例ではリソゾー

ムの数が増し、同時にリソゾーム内酵素活性の上昇が認められた。時々空胞をもつたリソゾームの出現もみられた。

これらの事実は軸索反応の際に前角細胞内において、酸性フォスファターゼ活性が上昇することを示している。この酵素活性の上昇は細胞内加水分解作用の亢進を示すかもしれないが、一方酸性フォスファターゼが軸索反応の際に、直接前角細胞内物質代謝に関与していることを全く否定し去るわけには行かない。